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Multishell Microspheres with Integrated Chromatographic and Detection Layers for Use in Array Sensors

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Not surprisingly, the recent interest in micrototal analysis systems has led to the development of numerous miniaturized liquid chromatography devices.[1,2] Most of these systems exploit developments in microfabrication to scale down conventional chromatographic instruments. Accordingly, emphasis here has been placed on minimizing sample volume, increasing sample throughput rate, and improving separation of analytes. Concurrently, there has been a move toward array-based sensing where the simultaneous response from a collection of low-selectivity sensing elements creates a diagnostic fingerprint response.[3−8] However, there are few, if any, prior works which combine microchromatographic technologies with array-based sensing concepts. Previously, we have reported the development of a novel optical sensor array platform consisting of polymer beads which are synthetically transformed into colorimetric sensing elements and then arranged in an array of wells etched in a silicon chip.[9−12] These bead-chip assemblies are housed within flow-cells, which are integrated with a combination of fluidic and optical components affording the near-real-time monitoring of solution-borne analytes. Prior demonstrations of this "Electronic Taste Chip" platform's utility have included measurements of pH, metal cations, simple sugars, biological cofactors, and serum antigens/antibodies.

Here we present new concepts for and demonstrate the utility of integrated chromatographic elements and generic detection functionalities for bead-based sensor array systems. Discrete separation and detection functionalities were created within beads using previously described[13,14] methodology for selective chemical modification of specific shell layers within individual beads. Likewise, temporal and stoichiometric control factors were used in conjunction with blocking agents, ligands, and indicator dyes to create chemically distinct regions within the beads. The first step in creating these functional bead systems involves the selective blocking of the active groups in the exterior regions of the beads. Next, the remaining active central core regions of the beads are sensitized to analytes through the immobilization of an appropriate optical detection scheme. Finally, after removal of the blocking layer on the beads' exteriors, the outer shells are functionalized with a ligand system. This process essentially creates microscopic, spherical chromatographic systems. For each bead, the ligand shell functions as a chromatographic[15] support, differentially hindering analyte progression through the polymer matrix, while the detection scheme localized at the core serves as an integrated detector element.

The utility of this new integrated chromatographic-detection concept is studied here in the context of generic metal cation detection experiments. In this example, alizarin complexone (ALZC), a compleximetric dye known to exhibit responses to a wide range of metal cations,[16] is used as the detector element.

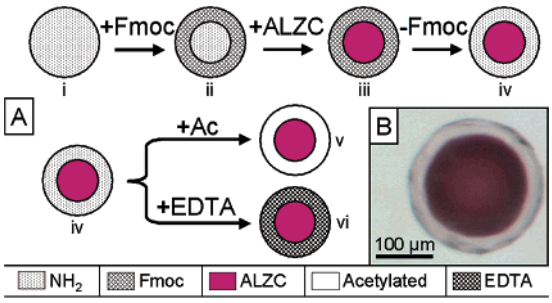

Figure 1. (A) Schematic showing the preparation of multishell beads with a common core (ALZC), but with different outer shells (v: acetylated; vi: EDTA). (B) Transmitted light image of a bead from batch iv. Batches v and vi appear nearly identical to batch iv, and are not shown.

Figure 1A displays schematically the synthesis of functional multishell beads. Key to the creation of distinctly heterogeneous regions within the amine-terminated polystyrene−poly(ethylene glycol) (TG-NH_2) beads is the ability to partially penetrate the resin in a radial manner with 9-fluorenylmethoxycarbonyl chloroformate (Fmoc). As demonstrated elsewhere,[13,14] this process allows for the creation of an external shell (within each bead) in which the amine sites are protected. The depth of this shell can be varied as a function of reaction time and Fmoc concentration. Accordingly, TG-NH_2 resin (i) is protected in this fashion, yielding resin with an exterior shell of protected amines (ii). Subsequent coupling of ALZC to ii results in beads with the complexone immobilized only within their cores (iii). Removal of the Fmoc protecting group then yields resin with an ALZC core and an exterior shell of free amines (iv). As such, the radial penetration of the Fmoc allows for a given chemical functionality to be inserted into the center of the resin, effectively bypassing the outer shell. Two aliquots of iv are individually treated with acetic anhydride and EDTA anhydride, respectively, yielding two batches with identical cores, but different exterior shells. While batch vi is functionalized with a strongly chelating EDTA shell, the amines in the exterior of batch *v* are capped, rendering the shell relatively inert with respect to metal cations.

Beads from batches v and vi were arranged in an Electronic Taste Chip array with each truncated pyramidal well hosting an individual bead, directing solution flow to the bead while allowing optical measurements to be made. The effective absorbance[9] of each bead was monitored vs time as various metal cation solutions were delivered to the flow cell. The chromatographic nature of these beads adds a new dimension to the data yielded by the Electronic Taste Chip platform, allowing for the extraction of multiple types of information from each bead's response to a given metal cation solution. For this demonstration, three such components of the beads' responses are defined as follows: (1) the *color change* of a bead is calculated by subtracting its initial effective absorbance value from its final effective absorbance value, (2) t_D is the time measured from the beginning of a bead's *color change* until the

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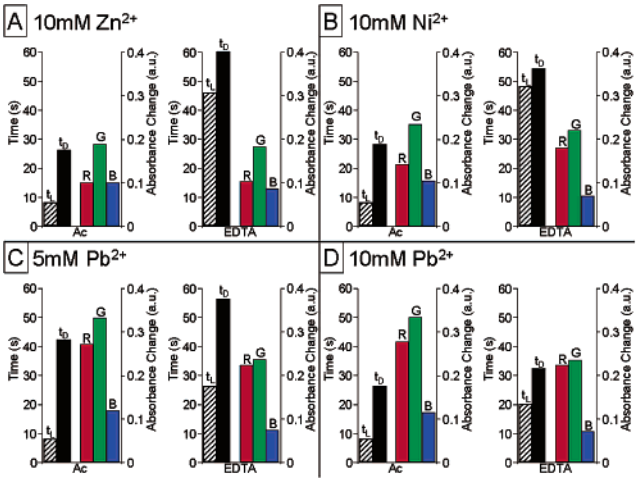

Figure 2. Graphical representation of multicomponent fingerprint responses yielded by functional multishell beads upon the introduction of (A) 10 mM Zn^{2+}, (B) 10 mM Ni^{2+}, (C) 5 mM Pb^{2+}, and (D) 10 mM Pb^{2+}. The t_L and t_D values of the beads' responses are shown in hatched and black bars, as indicated, and correspond to the left-hand axes. Red, green, and blue bars (measured on the right axes, labeled R, G, and B) display the beads' color changes in three channels as observed by a CCD camera. In each panel the beads are identified by their outer shells (Ac: acetylated; EDTA: immobilized EDTA).

bead has completed half of its *color change*, (3) t_L is the time required to penetrate the ligand shell as defined by the length of time prior to the observation of the *color change*.

Examples of the multicomponent responses are graphically summarized in Figure 2. Each of the four panels here included corresponds to the indicated metal solution and features two separate data sets associated with EDTA and acetylated outer shells. Interestingly, the individual components of each panel can be combined to form a fingerprint response showing unique characteristics for each of the studied metal cations. These reproducible data are well-suited for use with pattern-recognition algorithms. A comparison of panels C (5 mM Pb^{2+}) and D (10 mM Pb^{2+}) emphasizes the benefits of the increased dimensionality of the fingerprint response. While the *color changes* exhibited by the two bead types show little, if any, meaningful difference between the two concentrations, the t_D values of both beads, and the t_L values of the EDTA bead, differ significantly between the two concentrations. It is evident from these data that the final static colorimetric response (the *color change*) of the ALZC alone is insufficient for discriminating between the two concentrations of Pb^{2+}, and that the functional EDTA shells and the time domain have added to the array's capabilities. Conversely, in the cases displayed in panels A (10 mM Zn^{2+}) and B (10 mM Ni^{2+}) the t_D and t_L values of the beads differ only slightly between the two metals, while their *color changes* are distinctly different. For these cases, the colorimetric responses of the ALZC contribute more to the discrimination than do the temporal components of the response. Likewise, a comparison of panel D (10 mM Pb^{2+}) with either panel A (10 mM Zn^{2+}) or B (10 mM Ni^{2+}) demonstrates a situation in which both the temporal and colorimetric components differ between metals. That the t_L values of the acetylated (v) beads do not vary significantly between these four cases agrees well with the idea of an "inert" shell and highlights the chromatographic role provided by the EDTA functionality. Current studies involving a wider range of metal cations and a collection of different external ligands aim to further expand the utility of this new chemometric approach.

In conclusion, the initial demonstration of an array-based sensor composed of integrated chromatographic and detection elements has been completed. The new concepts described here allow for the batch production of multicomponent sensing ensembles whose chromatographic and detection functions can be chemically tailored to a given application by exchanging either of the integrated elements. Moreover, this work establishes a method for the facile generation of a collection of low-selectivity sensors based on a single detection scheme. Such complementary, overlapping elements are the building blocks of cross-reactive sensor arrays. The extension of this concept to include various other N-, S-, O-, P-based ligands, chelators, crowns, and cryptands should further enhance the scope and discriminatory capabilities of the outer shells, allowing for the creation of additional interesting "coordination chemistry" theme chips. Furthermore, with the appropriate components, this integrated technique should be readily applicable to other classes of analytes. Future efforts may exploit analogous components drawn from various branches of chromatography including molecular exclusion, ion exchange, and affinity chromatography.

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Supporting Information Available: Bead preparation, data collection, analysis, and reproducibility details (PDF). This information is provided free of charge via the Internet at http://pubs.acs.org.

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